

## EVALUATION OF SPERMATOPHORE COUNTS IN STUDYING MATING SYSTEMS OF LEPIDOPTERA

ROBERT C. LEDERHOUSE, MATTHEW P. AYRES,  
AND J. MARK SCRIBER

Department of Entomology, Michigan State University, East Lansing, Michigan 48824

**ABSTRACT.** Counts of spermatophores contained by field-collected females have been widely used to investigate mating behavior of Lepidoptera. We present new counts for *Papilio glaucus* L. females and reanalyze published data for this species to evaluate the often implicit assumptions of this technique. In addition, we relate spermatophore size and sequence to mating history of tiger swallowtail females captured in Wisconsin. Number of spermatophores per female increased with both wear class and capture date. Females that received small first spermatophores were significantly more likely to contain one or more additional spermatophores than those that received large first spermatophores. This suggests that more spermatophores per female result from inferior initial matings and not necessarily from male mating preference.

**Additional key words:** spermatophore size, multiple-mating probability, *Papilio glaucus*, Papilionidae.

Because one spermatophore is passed during each copulation in most Lepidoptera, and generally persists in recognizable form, counts of spermatophores contained in females have been used to infer aspects of mating systems in this taxon (Burns 1966, 1968, Taylor 1967, Pliske 1973, Ehrlich & Ehrlich 1978, Smith 1984). Burns (1968) documented the validity of these two assumptions and warned of potential biases from different aged samples. However, interpretations of spermatophore data are limited in additional ways that frequently have been ignored.

Mating histories of female tiger swallowtails, *Papilio glaucus* L., as revealed by spermatophore counts, probably are documented better than any other species of butterfly (Drummond 1984). Over much of its geographic range, *P. glaucus* females may be tiger-striped yellow like males, or dark mimics of the distasteful *Battus philenor* (L.) (Brower 1958). Spermatophore counts were analyzed to evaluate the role of sexual selection in maintaining this sex-limited color dimorphism in female adults (Burns 1966, Makielski 1972, Pliske 1972, Platt et al. 1984). Drawing on this literature and additional studies in our laboratory, we illustrate the strengths and limitations of using spermatophore count data.

### MATERIALS AND METHODS

Samples of *Papilio glaucus canadensis* females were collected from five adjacent counties in north-central Wisconsin during the flight period of the single generation in 1985. Of the 282 females collected, 152 were set up for oviposition; the other 130 were frozen until dissected.

Females were assigned to a condition class either when set up or dissected. We classified 78% of the females for wing wear within a four-day period, which reduced potential bias due to changing standards by the classifier during the flight season as discussed below. Females were carefully dissected so that both number of spermatophores and their relative position in the bursa copulatrix were determined (Drummond 1984). Since the spermatophore of an additional mating forces the previous spermatophore forward in the saclike, posteriorly opening bursa copulatrix, and since spermatophores change from creamy white to yellow and collapse with time, accurate sequences of spermatophore deposition were determined for 98% of collected females. Volumes of the nearly spherical *P. g. canadensis* spermatophores were estimated by averaging the longest and shortest diameters and calculating the volume of such a sphere. Spermatophore dimensions were not measured for 52 females.

#### RESULTS AND DISCUSSION

**Reliability.** Spermatophore counts are generally reliable as a measure of female mating history (Burns 1968, Lederhouse 1981, Drummond 1984). Deviations from the one copulation-one spermatophore assumption are rare. Only occasionally will *Papilio* males not pass a spermatophore during coupling, and we have detected only one case of two spermatophores being passed during a single copulation ( $n = 226$  hand-pairings of *Papilio*). This exceptional case occurred during a prolonged coupling lasting over 24 h. The same sample also contained one female that laid viable eggs but contained no detectable spermatophore. She did have seminal material in her bursa. Spermatophores are persistent in swallowtail females; even females that have been maintained for 20–30 days in the laboratory have obvious spermatophores. However, spermatophores may disintegrate rapidly in the lower Lepidoptera (Taylor 1967) where the spermatophore lacks chitin. Also, spermatophores are gradually absorbed in females of a variety of higher Lepidoptera (Burns 1968, Ehrlich & Ehrlich 1978). This is particularly true for species where females use nutrients contributed by males at copulation for egg production (Boggs & Gilbert 1979, Boggs 1981). Therefore, it seems prudent to verify the one copulation-one spermatophore relation for each species for which spermatophore counts are used.

**Ageing females.** Even when spermatophore counts reliably indicate number of copulations, there remain a variety of factors that must be considered in evaluating such data in the context of mating behavior and sexual selection. The difficulties chiefly involve controlling for female age and spermatophore quality.

It is logical to assume that older females should carry more sper-

TABLE 1. Mean number of spermatophores contained by *Papilio glaucus canadensis* females from Wisconsin in relation to wing condition and date of capture.

Condition	June 1985			
	6	13	20	27
Fresh	1.00	1.00	1.50	1.63
Slightly worn	1.20	1.13	1.55	1.85
Intermediate	1.56	1.61	1.84	2.33
Very worn	2.00	2.10	2.12	2.62
Mean	1.48	1.53	1.89	2.30
Sample size	25	108	79	70

matophores than younger females. However, it is difficult to age field-collected females accurately. Recapture rates of marked females are usually too low to provide known age samples (Lederhouse 1982). Wing wear is an estimate of age, but may reflect the quality of life (encounters with predators, inclement weather, or other factors; Lederhouse et al. 1987) as much as its quantity. By their nature, estimates of age are subjective and may vary from investigator to investigator. Since representatives of all age classes are not present throughout a flight season, our experience suggests a tendency to overestimate age early in a generation when very worn individuals are scarce and underestimate it late in a generation when very fresh individuals are rare. Nevertheless, spermatophore numbers carried by females of a variety of species have been shown to increase with estimates of age such as wing wear (Burns 1968, Lederhouse 1981, Drummond 1984, Lederhouse & Scriber 1987). This is illustrated within and across four sampling days for *P. g. canadensis* females (Table 1).

Accurate comparisons of samples rely on similar age structures or the ability to control for age structure in analysis. However, female age structures of natural butterfly populations are largely unknown. Spermatophore counts necessarily underestimate lifetime mating frequency. Sampling removes females at an artificial point. Once sampled, a female that would have remated the next day, and perhaps again the following week, becomes equivalent to a female that would never have remated. Since females may be singly-mated as a result of their mating system or their young age, studies indicating female monogamy (Wiklund 1977, 1982, Wiklund & Forsberg 1985) must give age estimates for their samples.

Comparisons of mating histories for different female morphs within a population must control for female age. Pliske (1972) questioned the importance of sexual selection in maintaining the frequency of the yellow female phenotype in a Florida population of *P. glaucus* because

he found no difference in number of spermatophores carried by yellow or dark females. Our reanalysis of Pliske's data indicates that his sample of *P. glaucus* females cannot address effects of sexual selection on differential remating of the two female morphs because females of both morphs had mated too few times for a differential to be detectable. Either females in his sample were too fresh (young) for many to have mated more than once, or multiple-mating was rarer in central Florida *P. glaucus* populations (Pliske 1972, 1973) than elsewhere (Burns 1966, Makielski 1972, Platt et al. 1984, Lederhouse & Scriber 1987).

**Spermatophore quality.** Spermatophore quality may influence the frequency with which a female mates. A common assumption is that more spermatophores indicate superior mating, which is based on the supposition that all spermatophores are equal. Yet young or frequently-mating males produce smaller than average spermatophores (Sims 1979, Svard & Wiklund 1986). Spermatophore volumes in early season *P. g. canadensis* females suggest a bimodal distribution (Fig. 1). Hand-pairing in our laboratory indicates that the larger spermatophores (about 7 mm<sup>3</sup>) result from males' first matings and the smaller ones (about 4 mm<sup>3</sup>) from subsequent matings (Lederhouse, Ayres & Scriber in prep.). Spermatophore size distribution from a late-season sample shows a significant increase in frequency of smaller spermatophores compared with the early-season sample (Fig. 1; Kolmogorov-Smirnov test,  $P < 0.005$ ). Small spermatophores may result in lower egg fertility or more rapid fertility declines (Lederhouse & Scriber 1987). Size of spermatophores is therefore an important aspect of quality that should be considered.

Since stretch receptors in the female's bursa copulatrix may determine female receptivity (Sugawara 1979), smaller than average spermatophores might produce a shorter mating refractory period or none at all. Indeed, a multiway contingency analysis of the probability of *P. g. canadensis* females carrying multiple spermatophores demonstrates a significant effect of spermatophore size, in addition to date of capture and female condition class (Fig. 2, Table 2). Only 45% of 160 females that received a large first spermatophore ( $>4$  mm<sup>3</sup>) carried more than one spermatophore compared with 63% of 70 females that had a small first spermatophore ( $\leq 4$  mm<sup>3</sup>). This difference is significant ( $\chi^2$ ,  $P = 0.002$ , Table 2) although both samples had similar age distributions as indicated by wing-wear classes ( $\chi^2$ ,  $P > 0.25$ ). This suggests that females mated again to replace a small spermatophore. We observed a similar relation for *P. g. glaucus* from an Ohio population (Lederhouse & Scriber 1987). Of 164 females that received a large first spermatophore, 45% had mated more than once compared with 67% of 165 females



## FREQUENCY (%)

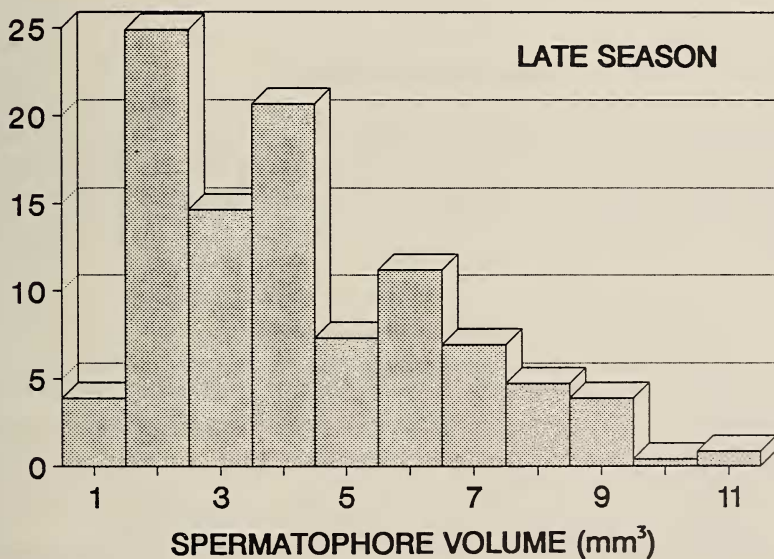
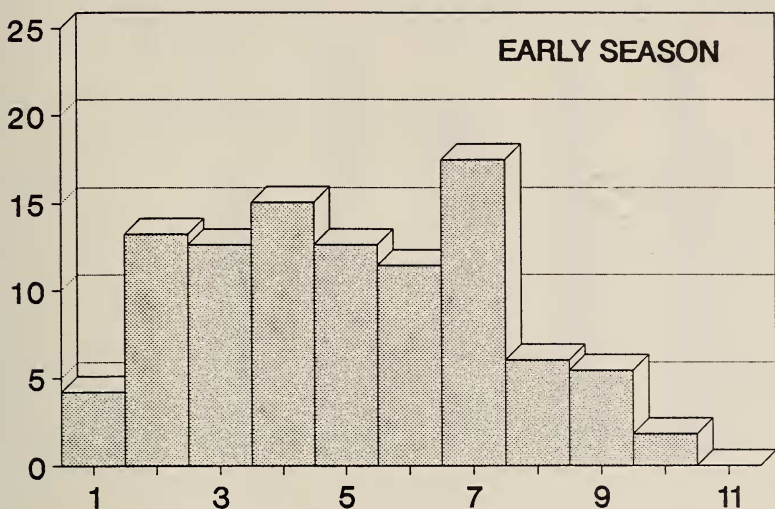


FIG. 1. Size distribution of Wisconsin *Papilio glaucus canadensis* spermatophores found in early-season females (captured 6–13 June 1985) and in late-season females (captured 20–27 June 1985). N = 166 and 232 spermatophores, respectively.

TABLE 2. Results of multiway contingency analysis (SAS CATMOD) of the probability of multiple-mating in *P. g. canadensis*. The null hypothesis tested for each source effect was that proportion of remated females (carrying 2 or more spermatophores) did not differ between categories of the independent variable. A graphical representation of the analysis is in Fig. 2.

Source	df	Chi-square	P
Condition	2	32.4	0.0001
Spermatophore size	1	10.2	0.002
Date of collection	1	5.6	0.018
Condition × date	2	4.7	0.10
Other interactions	1-2	<3.0	>0.22

that had a small first spermatophore ( $\chi^2 = 15.1$ ,  $P < 0.0001$ , where condition class and year were other sources of variation in the model).

Male *P. glaucus* generally emerge before females (Berger 1986), which provides time for them to reach full sexual maturity before copulation. Nevertheless, some small spermatophores carried by early females could come from immature males because emergence curves of the sexes do overlap (Berger 1986). The significant relation between date of capture and probability of remating (Tables 1, 2) may result from an increased proportion of females mating with previously mated males later in the generation. Since size of spermatophore passed by a male decreases with additional matings in *P. g. canadensis* (Lederhouse, Ayres & Scriber in prep.) and proportion of males that had mated at least once appears to increase later in the generation (Fig. 1), later mating females were more likely to receive an insufficient spermatophore and mate again after a short refractory period. Late in the generation, it was not uncommon for even fresh females to carry three or four smaller than average spermatophores.

Even the size of a spermatophore may be a poor indicator of its quality (Greenfield 1983, Jones et al. 1986). In our study of 1985 *P. g. canadensis* females, we could detect no significant relation between spermatophore size and percent egg hatch, despite wide variation in egg hatch (range 0.0-97.5%,  $\bar{x} = 58.5$ ,  $n = 23$ ). Similar-sized spermatophores may vary in relative proportions of different constituents (Marshall 1982, Alcock & Hadley 1987, Marshall & McNeil in press). This may be particularly important for those species where the spermatophore and associated secretions contribute to the nutrient pool available to females for reproduction. Selection could favor male sperm delivery strategies that treat females of different reproductive value differently (Boggs 1981), or that fool a female with large but inexpensive, low quality spermatophores.

Persistence of courtship is related to mating history in some lepi-

## PERCENT MULTIPLE MATINGS

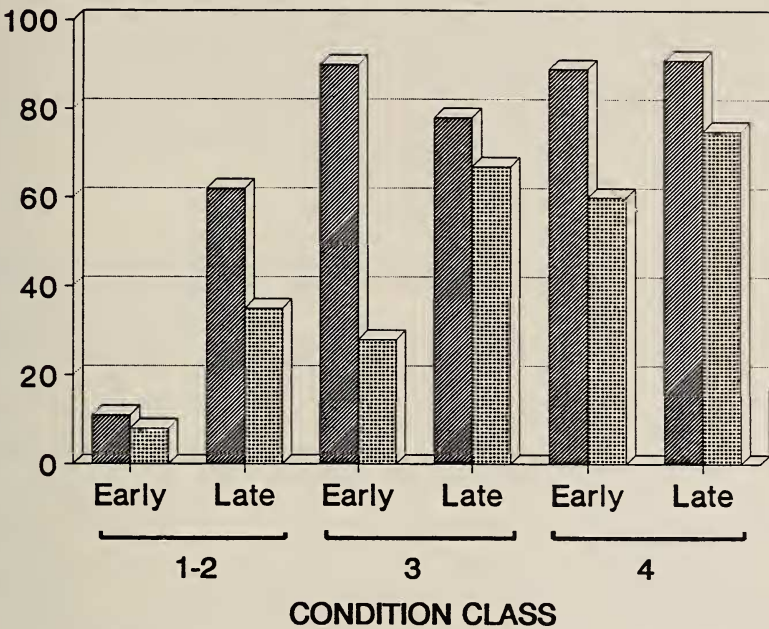


FIG. 2. Probability of remating in Wisconsin *Papilio glaucus canadensis* females as influenced by first spermatophore size, date of collection, and condition class. In 6 of 6 comparisons females receiving a small 1st spermatophore (hatched bars) were more likely to remate than females receiving a large 1st spermatophore (stippled bars). Associated contingency tests are in Table 2. Early-season females were captured 6–13 June and late-season females 20–27 June 1985.

dopteran species (Rutowski 1979, 1980). High selectivity by either a male or a female could lead to passing of a larger than average spermatophore followed by a longer than average mating refractory period. Thus, preferred females might carry fewer but larger spermatophores on average. Larger *Dryas julia* Fabr. females received larger spermatophores (Boggs 1981). Less selective males might mate more frequently but pass smaller than average spermatophores. Less selective females might receive smaller spermatophores, remate at shorter intervals, and as a result carry more spermatophores on average. Such potential results run counter to the logic of Burns (1966) and others.

These various factors do not invalidate spermatophore counts but suggest that more care must be taken in interpreting count data. Counting spermatophores remains a valuable tool, but count data must be integrated with that of other techniques to yield an accurate appraisal of mating behavior in Lepidoptera.

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